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Eline T. Luning Prak

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Commentary

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Restoring balance to B cells in ADA deficiency

Eline T. Luning Prak

Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

It is paradoxical that immunodeficiency disorders are associated with autoimmunity. Adenosine deaminase (ADA) deficiency, a cause of X-linked severe combined immunodeficiency (SCID), is a case in point. In this issue of the *JCI*, Sauer and colleagues investigate the B cell defects in ADA-deficient patients. They demonstrate that ADA patients receiving enzyme replacement therapy had B cell tolerance checkpoint defects. Remarkably, gene therapy with a retrovirus that expresses ADA resulted in the apparent correction of these defects, with normalization of peripheral B cell autoantibody frequencies. In vitro, agents that either block ADA or overexpress adenosine resulted in altered B cell receptor and TLR signaling. Collectively, these data implicate a B cell-intrinsic mechanism for alterations in B cell tolerance in the setting of partial ADA deficiency that is corrected by gene therapy.

Complete adenosine deaminase (ADA) deficiency causes hepatic, skeletal, neurologic, and immunologic defects and results in failure to thrive, recurrent severe infections, and ultimately death if untreated. The profound manner in which ADA deficiency causes immunologic dysfunction has been studied since its discovery in the 1970s (1). The ADA enzyme deaminates adenosine to inosine, and it also deaminates 2'-deoxyadenosine (dATP) to deoxyinosine (2). Consequently, ADA deficiency results in elevated levels of adenosine and dATP, and accumulation of these metabolites is toxic to lymphocytes (2). In the thymus, where the normal process of T cell development results in extensive cell death, extracellular DNA and high levels of deoxynucleoside kinases together create a persistent source of dATP (3). In the absence of ADA, increased intracellular dATP levels interfere with cellular metabolism, promote mitochondrial cytochrome *c* release, and ultimately cause apoptosis (3–5). Thus, ADA knockout mice suffer from pronounced T cell defects. Importantly, they also exhibit defects in splenic B cell subsets and architecture, along with decreased levels of B cell proliferation and activation (6, 7).

Immune imbalance in ADA deficiency

A model for how ADA deficiency affects the immune system is presented in Figure 1.

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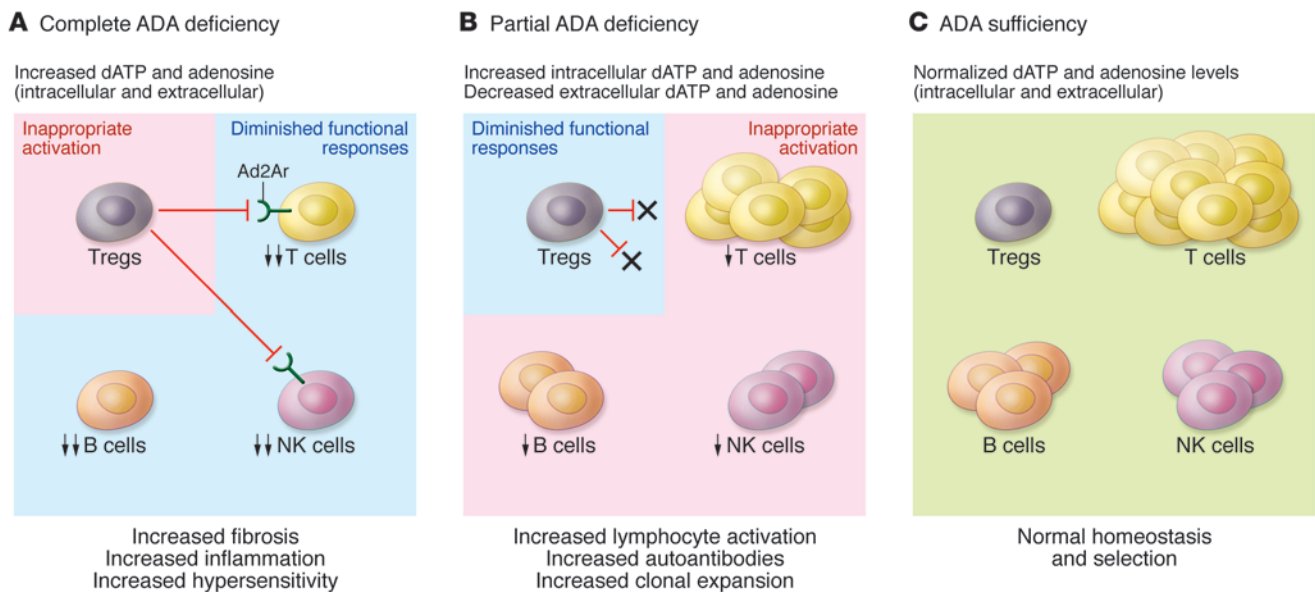
In the complete absence of ADA, there is metabolic toxicity, thymic hypoplasia, and profound T, B, and NK cell lymphopenia. The extracellular accumulation of dATP and adenosine alters lymphocyte signaling pathways and serves as a danger signal that can promote phagocytosis and inflammation (8). In this context, some innate immune cells and other pathways that are less sensitive to ADA deficiency may operate chronically rather than acutely, producing the fibrotic and inflammatory lesions seen in humans and ADA knockout mice (reviewed in ref. 2). In the setting of partial ADA deficiency, more of the lymphocytes survive, providing an additional layer of complexity for immune dysregulation. Patients with milder forms of ADA deficiency can develop immunopathology including type 1 diabetes, autoimmune thrombocytopenia, hemolytic anemia, and hypothyroidism as well as allergies and other hypersensitivities (9). dATP released by stimulated T cells can be taken up by nucleoside transporters and promote activation and proliferation of neighboring cells (8). Intriguingly, Tregs express high levels of the ectoenzymes CD39 (which produces AMP from ADP or ATP) and CD73 (which converts AMP to adenosine, ref. 10). Extracellular adenosine produced by Tregs can engage the inhibitory adenosine 2A receptor on T and NK cells (8). With enzyme replacement therapy, extracellular (but not intracellular) adenosine levels fall, reducing the efficacy of Treg-mediated T cell inhibition (10). ADA-deficient patients may also be lymphopenic, which can be accompanied by elevated levels of B

lymphocyte stimulator (BLyS; also known as BAFF), a TNF superfamily member that influences the stringency of peripheral B cell selection (reviewed in ref. 11). Thus, the immune system is precariously balanced in ADA deficiency, with severe defects in lymphocyte production and reliance on innate and inflammatory pathways for immune defense on the one hand, and imbalanced lymphocyte homeostasis, immunoregulation, and signaling on the other.

B cell tolerance defects in ADA deficiency

In this issue of the *JCI*, Sauer et al. studied the antibody repertoires of peripheral B cell subsets in three patients with ADA deficiency, two of whom were receiving enzyme replacement therapy (12). Given the tenuous balance of the immune system in ADA deficiency, it is perhaps not surprising that they found increased proportions of autoreactive B cells in these patients. The authors describe an abnormal antibody repertoire in transitional B cells, the earliest bone marrow emigrants to circulate at significant levels in the peripheral blood. They also found that cells that had progressed to a later developmental stage, called mature naive cells, exhibited an increased frequency of autoreactive clones, suggestive of a second defect in B cell tolerance.

How do these B cell tolerance defects arise? The authors propose that the abnormal repertoire in the transitional B cell compartment reflects a central (bone marrow) B cell tolerance checkpoint defect. Their in vitro data suggest that ADA substrates can interfere directly with B cell receptor (BCR) and TLR signaling and resultant B cell activation. Similarly, it was previously shown that in vitro, adenosine-treated murine B cells exhibit reduced NF- κ B activation in response to BCR or TLR ligation (13). Altered signaling could fail to trigger the central B cell tolerance mechanisms of receptor editing or apoptosis, reducing the counterselection of autoreactive clones. Consistent with this hypothesis, other patients with primary immunodeficiencies (including those with IRAK-4 and MYD88 deficiencies, in which TLR signaling is compromised) have similarly affected B cell com-

**Figure 1**

Restoring immune balance in ADA deficiency. **(A)** In the absence of ADA, intracellular and extracellular levels of the ADA substrates dATP and adenosine increase, and numbers of B, T, and NK cells are drastically reduced. Immune cells are hypofunctional except for Tregs, which have higher levels of ectoenzymes that can metabolize purinergic substrates to adenosine. Extracellular adenosine, in turn, can engage inhibitory adenosine 2A receptors (Ad2Ar) on NK cells and T cells. T, NK, and B cell functional responses are diminished (blue background). There is increased chronic innate immune stimulation, leading in part to fibrosis, inflammation, and hypersensitivity reactions. **(B)** In the setting of ADA enzyme replacement therapy, extracellular levels of adenosine and dATP are markedly reduced, whereas intracellular levels are still elevated. The reduced levels of extracellular adenosine diminish the inhibitory activity of Tregs. There is still moderate lymphopenia, but inappropriate lymphocyte activation due to altered TLR and BCR signaling and tolerance checkpoint defects (pink background), resulting in autoimmune manifestations. **(C)** After successful gene therapy, intracellular and extracellular levels of adenosine and dATP normalize, lymphocyte numbers increase, and proper homeostasis and selection mechanisms are restored (green background).

partments (reviewed in ref. 14). Also consistent with a central tolerance defect was the increased frequency of κ light chain usage in transitional B cells observed in the ADA-deficient patients (82% were κ^*), suggestive of reduced light chain receptor editing (15). However, a bone marrow B cell tolerance checkpoint defect in ADA deficiency is not directly proven by these data and is inconsistent with the comparatively normal bone marrow B cell subset composition in ADA knockout mice (2). An alternative explanation is suggested by the finding of skewed light chain usage in transitional cells, with an increased frequency of a specific chain called $\lambda 2-23$. These findings could reflect abnormal selection for B cells that recognize one or more specific autoantigens and/or altered survival of autoreactive B cells in the transitional pool, rather than a tolerance defect in the bone marrow.

The altered autoantibody repertoire in mature naive B cells could arise secondary to altered T cell/B cell collaboration, as proposed by Sauer et al. (12). For example, if Treg inhibition of activated T cells is reduced during ADA enzyme replacement

therapy (10), perhaps there is a relative overabundance of inappropriate T cell help for B cells. A condition of excess T cell help is reminiscent of chronic graft-versus-host disease in mice, in which B cell tolerance to nuclear antigens can be broken solely by the transfer of alloreactive CD4⁺ T cells, which interact with host B cells (16). On the other hand, one of the three patients had not received enzyme replacement therapy, making the reduced Treg function harder to explain in this patient, and T cell counts were presumably quite low in all three patients prior to gene therapy, calling into question where the excess T cell help would have come from (12). Furthermore, the manner in which mature naive B cells use T cell- or TLR-derived signals for survival or selection is not yet well understood in humans. Enhanced survival of autoreactive mature naive B cells could also result from increased BlyS levels, but these were not measured in the present study.

Restoring balance with gene therapy

A remarkable finding of this study is how effectively gene therapy re-equilibrated the

B cell compartment: absolute B cell counts normalized, there was a more normal B cell subset composition, the use of intravenous immunoglobulin was discontinued, patients mounted antibody responses to vaccination, and they did not exhibit autoimmune phenomena. Strikingly, gene therapy also reduced the frequency of autoreactive B cells and resulted in the normalization of serum autoantibody levels in all three patients (12). Thus, the restoration of immunologic function and reduction of autoimmunity after gene therapy suggests that intracellular ADA is critical for maintaining the proper balance of function, survival, and selection in the B cell compartment. Perhaps more subtle abnormalities of adenosine metabolism contribute to some autoimmune diseases. The perturbations in B cell selection in ADA deficiency will continue to provide a fertile area for future investigations into fundamental aspects of human lymphocyte biology.

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Address correspondence to: Eline T. Luning Prak, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. Phone: 215.746.5768; Fax: 215.573.6317; E-mail: luning@mail.med.upenn.edu.

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Telomere stability and carcinogenesis: an off-again, on-again relationship

Jennifer J. Wanat and F. Brad Johnson

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA.

Previous studies in mice have demonstrated antagonistic effects of telomerase loss on carcinogenesis. Telomere attrition can promote genome instability, thereby stimulating initiation of early-stage cancers, but can also inhibit tumorigenesis by promoting permanent cell growth arrest or death. Human cancers likely develop in cell lineages with low levels of telomerase, leading to telomere losses in early lesions, followed by subsequent activation of telomerase. Mouse models constitutively lacking telomerase have thus not addressed how telomere losses within telomerase-proficient cells have an impact on carcinogenesis. Using a novel transgenic mouse model, Begus-Nahrman et al. demonstrate in this issue of the *JCI* that transient telomere dysfunction in telomerase-proficient animals is a potent stimulus of tumor formation.

Telomeres and telomerase in cancer

Telomeres have a mixed reputation when it comes to cancer. On the one hand, the chromosome-protective functions of telomeres (capping) can be lost with the shortening of telomeres that accompanies cell division, which in turn can limit cell proliferation. When telomeres become critically short and uncapped, they lose their ability to disguise the linear ends of chromosomes from the DNA damage and checkpoint

response machinery, which — depending on cell context — leads to cell-cycle arrest (senescence) or cell death (1). Thus loss of telomere reserves may stymie a clone of incipient cancer cells before it can give rise to a significant tumor. On the other hand, rare cells that have sufficiently inactivated their checkpoint response machinery (e.g., via mutation) may continue to divide despite telomere losses. In the case of cultured human fibroblasts, inactivation of the p53 and p16/Rb pathways enables bypass of senescence (2). Uncapped telomeres are prone to recombination, including ligation to other uncapped telomeres, yielding dicentric chromosomes that, fol-

lowing a tug-of-war at mitosis, generate nondisjunction events or internal chromosome breaks. Cycles of these so-called breakage–fusion–bridge events drive gene sequence and copy number changes leading to cell dysfunction and death, which, in human fibroblasts that have bypassed senescence, is called crisis. But they also provide fertile ground from which rare variants can emerge to form tumors (3). Therefore, a question of fundamental importance is whether telomere losses play net inhibitory or stimulatory roles in carcinogenesis. A correlative question of greater practical importance is whether inhibition of the telomere-lengthening enzyme telomerase is likely to benefit cancer patients.

In humans, telomerase activity is under strict control, in part via epigenetic regulation of genes encoding its components, including the TERT catalytic protein and the TERC template RNA (4). Although telomerase can be detected in the progenitor cells of highly proliferative tissues, its activity is nonetheless insufficient for preventing age-related decreases in telomere lengths. Thus, telomeres would be expected to shorten in a runaway premalignant clone of cells. Indeed, premalignant lesions are

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